<Original Articles>Effect of Total Parenteral Nutrition on Liver Mitochondrial Function in Mature Rats

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Effect of Total Parenteral Nutrition on Liver Mitochondrial Function in Mature Rats

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Abstract

To evaluate the effects of TPN on the hepatic function, the changes in hepatic energy charge levels, oxidative and phosphorylative activities of mitochondria and serum transaminase were studied, using male Sprague-Dawley rats 240 to 250 g in weight. The rats were randomized into three groups. The first group (TPN–V group, n=6) was infused with TPN solution via the right jugler vein. The number of calories of TPN solution infused daiary was adjusted to provide each rat with 80 kcal/kg/day on the 1st day, 160 kcal/kg/day on the 2nd day and 240 kcal/kg/day on the 3rd day. After the 4th day, 240 kcal/kg/day was given to both groups. The second group (TPN–G group, n=5) was infused with the same solution via an intragastric route and was given the same calories as the TPN–V group. The third group (control group, n=6) was given a chow diet with the same calories as the TPN group. At the 13th day, all groups were sacrificed, and the hepatic energy charge (EC) and phosphorylation rate (PR) of hepatic mitochondria were measured, and liver function tests were done. PR was 101.2±5.0 nmol/mg protein/min in control group, 120.8±2.7 in TNP–G group and 136.5±6.2 in TPN–V group. EC was 0.906±0.006, 0.889±0.008, 0.831±0.010, respectively. The liver function tests of all group were normal.

In both TPN groups, despite evidence that liver function tests were normal, enhanced michodrial phosphorylative activity was observed during the early stage of TPN. The mitochondrial enhancement in the TPN–G group was smaller than that in TPN–V group. This result suggested that TPN places a load on liver mitochondria and that long term TPN may induce hepatic failure.

Introduction

Total parenteral nutrition (TPN) is frequently given to undernorished patients to improve nutritional status. On the other hand cholestatic jaundice and hepatic dysfunction have been observed in

Key words: TPN, Liver dysfunction, Energy metabolism, Mitochondrial phosphorylative activity.
索引語：TPN，肝機能異常，エネルギー代謝，肝ミトコンドリア酸化的リン酸化能

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association with TPN in both children and adults. Hepatic dysfunction, especially jaundice and elevated transaminase levels, is most commonly observed in the pediatric age group[6,18,23]. Long term TPN is known to cause cholestatic hepatic dysfunction, which often induces hepatic failure. But, despite many studies, the etiology of this hepatic malfunction has yet to be clarified.

We have studied the changes of hepatic energy charge and mitochondrial phosphorylative activties of the living body under metabolic overload. Of the many indicating factors of the hepatic falure, the derangement of the hepatic energy metabolism is perhaps the most critical one in hepatic failure[37]. In this study, we attempted to evaluate the effects of TPN on hepatic energy charge and mitochondrial function using rats, to clarify the relation between mitochondrial function, TPN administration and TPN route.

Materials and Methods

Male Sprague-Dawley rats 240 to 250 g in weight were used for this study. The rats were housed individually in metabolic cages. The rats were assigned to three groups: A) intravenous TPN (TPN-V, n=6); B) intragastric TPN (TPN-G, n=5); C) oral diet fed (control, n=6).

Animals were anesthtized by injecting i.p. pentobarbital at a dose of 30 mg/kg body weight. Rats in all groups had silicon catheter (ID 0.5 mm, OD 1.0 mm, Dow Corning Corp., Japan) inserted via the external juglar vein. In TPN-G group, gastrostomy was done to insert the same sized catheter into the stomach. Each rat was then attached to a harness with a stainless-steel spring. The catheter was passed through the harness and connected to a swivel.

The TPN groups were continuously infused by infusion pump with a TPN solution consisting of dextrose supplemented with appropliate electrolytes, 10% amino acid, multivitamines and trace elements. The composition of the infusion solution is shown in Table 1a. Both intravenous and intragastric fed rats were infused with approximately 72 ml of the TPN diet, which provided 240 kcal/kg/day. To avoid acute liver dysfunction, the number of calories of TPN solution infosed daia-

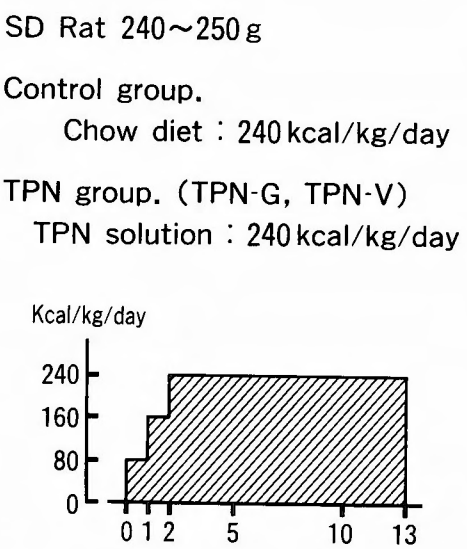

Fig. 1 Protocol for TPN

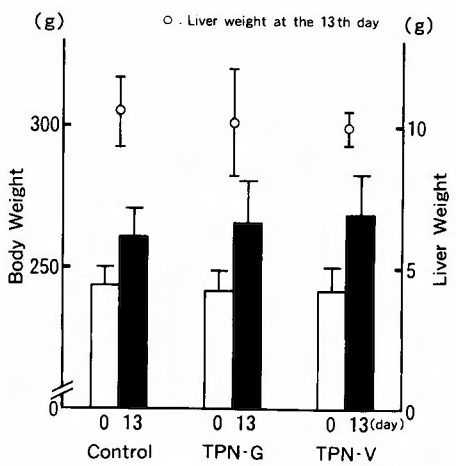

Fig. 2 Weight changes during experimental period.

Table 1a Components of TPN solution

Glucose	20 % w / v
Amino acid [1]	1.39 % w / v
Na	34.8 mEq / l
K	20.9 mEq / l
Cl	16.0 mEq / l
Ca	5.6 mEq / l
SO_4	5.6 mEq / l
Acetate	34.8 mEq / l
Gluconate	5.6 mEq / l
Trace elements [2]	1.67 ml / l
Vitamines [3]	1.67 ml / l
Total cal : 0.83 Kcal / ml	
Cal / N : 180	

[1] Amino acid (Moripron-F, Morishita Pharmaco. Corp. Osaka, Japan)
[2] Trace elements (TM-4, Morishita Pharmaco. Corp. Osaka, Japan : zinc chloride, 1.5 mg / ml ; cupric chloride, 0.15 mg / ml ; Fe, 1.0 mg / ml ; I, 0.075 mg / ml)
[3] Vitamines (MVI-12, SS Pharmaco. Corp. Osaka, Japan)

Composition of amino acid (200 ml)

L-isoleucine	1.12 g
L-leucine	2.50 g
L-lysine	2.48 g
L-methionine	0.70 g
L-phenylalanine	1.87 g
L-threonine	1.30 g
L-tryptophan	0.26 g
L-valine	0.90 g
L-alanine	1.24 g
L-arginine	1.58 g
L-aspartic acid	0.76 g
L-cistain	0.20 g
L-glutamic acid	1.30 g
L-histidine	1.20 g
L-proline	0.66 g
L-serine	0.44 g
L-tyrosine	0.07 g
Amino phosphate	2.14 g

MVI-12 (Total 10 ml)

Vit A	3300 IU
Ergocalciferol	200 IU
DL-tocopherol	10 mg
Thiamin HCL	3 mg
Riboflavin	3.6 mg
Pyrydoxine	4 mg
Niacinamide	40 mg
Panthenol	15 mg
Ascorbic acid	100 mg
Vit B_{12}	5 μg
Folic acid	200 μg
Biotin	60 μg

Table 1b Components of rat chow

Fibers	3.3%
Fats	4.8%
Proteins	27.5%
Soluble none-nitrogen	49.0%
Minerals	8.4%
Water	7.0%

Total cal : 3.72 Kcal/g

ly was increased gradually over the first three days (Fig. 1). The control group was infused normal saline solution at a continuous rate (3 ml/h) and fed standard chow (F-2, Funahashi Farms, Chiba, Japan) which provided caloric levels similar to the TPN group (Table 1b). Body weights were recorded on the first day and last day of the feeding period. All rats had access to water ad libitum.

On the 13th experimental day, rats were again anesthetized with ether and recieved laparotomy. Blood was drawn by aortic puncture for determination of serum glucose, bilirubin, GOT, GPT, ALP, NEFA, LAP, total protein, and total cholesterol.

About 0.5 g of the liver tissue was freeze-clamped in situ with stainless-steel tongs pre-cooled in liquid nitrogen for the assay of adenine nucleotides, and the remaining liver tissue was used for the assay of hepatic mitochondrial activity and histological analysis.

The frozen tissues were weighed and homogenized in an ice-cold solution of 6% (w/v) perchloric acid. The extract was then centrifuged at 10,000×g for 5 min at 0–4°C. The amounts of adenosine triphosphate (ATP), adenosine diphosphate (ADP), and adenosine monophosphate (AMP) were measured by high-performance liquid chromatography. Energy charge was

Table 2 Liver function test

	Control (n = 6)	TPN-G (n = 5)	TPN-V (n = 6)
T. Bil (mg/dl)	0.19±0.01	0.16±0.02*	0.20±0.00
GOT (U)	76.3±3.3	77.0±2.5	77.5±5.1
GPT (U)	25.0±1.1	21.0±1.5**	14.1±1.2***, ****
Al-p (U)	27.9±2.3***	19.3±1.3	23.1±3.9
NEFA (μEq/l)	0.52±0.03	0.62±0.07	0.48±0.02
LAP (U)	162.1±8.1	173.8±8.3	170.0±5.3
TP (g/dl)	5.7±0.1	5.8±0.1	6.0±0.2
T-Cho (mg/dl)	60.2±2.2	66.2±2.8	67.3±6.2
BS (mg/dl)	166.0±3.4*	157.6±4.9	141.7±10.7**

Values are Mean ± SEM
* $P < 0.05$, as compared to TPN-V group
** $P < 0.05$, as compared to control group
*** $P < 0.005$, as compared to TPN-G group
**** $P < 0.001$, as compared to control group

caluculated according to the formula of Atokinson[33]:

Energy charge=(ATP+1/2ADP) / (ATP+ADP+AMP).

Liver mitochondria were prepared by the method of Ozawa et al.[31,32]. Oxygen consumption was measured polarographically with a rotating electrode, using glutamate as substrate. The concentration of mitochondrial protein was determined by the method of Lowry et al.[38].

Nitrogen balance was determined for each animal on Day 12 of the experimental diets by micro Kjeldahl analysis of urine and feces for total nitrogen.

Statistical significance was determined by Student's t-test, and p values <0.05 were considered as significant.

Results

All rats gained weight and were clinically fit throughout the experimental period. After 13 days of feeding TPN solution or standard diet, the weight gaines of each group are as shown in Fig. 2. There was no significant difference in liver weight between groups. The weight gain of TPN group tended to be larger than that of the other groups, no significant differences was seen. The nitrogen balance of all groups was positive, and there is no significant difference in nitrogen balance between three groups.

Table 2 shows the results of liver function test. The liver function testsof all groups were normal.

The changes in concentration of adenine nucleotides and hepatic energy charge were shown in Table 3. In the TPN–V group, EC was 0.831 ± 0.010 and decreased markedly. In the TPN–G group EC was 0.889 ± 0.008, and 0.906 ± 0.006 in control group. Ther was a significant difference between the TPN–V group and other two groups ($p<0.05$). EC of the TPN–G group was less than that of the control group, but not significantly so.

The findings on the respiratory control rate (RC), State 3 respiratory rate, ADP/O and phosphorylation rate in isolated liver tissue are shown in Table 4. The PR in control group was 101.2 ± 5.0 and was not enhanced; 120.8 ± 2.7 in the TPN–G group; 136.5 ± 6.2 in the TPN–V

Table 3 Changes in adenine nucleotides and energy charge

	Control (n = 6)	TPN-G (n = 5)	TPN-V (n = 6)
Adenine nucleotide (μmol/g wet tissue)			
ATP	3.52±0.14	3.06±0.19	3.79±0.57
ADP	0.60±0.08	0.53±0.04	1.10±0.23*, **
AMP	0.25±0.04	0.15±0.01	0.34±0.08*
Total	4.37±0.21	3.74±0.20	5.24±0.76
Energy charge	0.906±0.006	0.889±0.008	0.831±0.010*, **

Results given are means ± SEM with n values in parentheses
Total = ATP + ADP + AMP ; Energy charge = (ATP + 1/2 ADP) / (ATP + ADP + AMP)
* $p < 0.05$, as compared to control group
** $p < 0.05$, as compared to TPN-G group

Table 4 Changes in liver mitochondrial function

	Control (n = 6)	TPN-G (n = 5)	TPN-V (n = 6)
RC	6.03 ± 0.93	4.88 ± 0.24	5.56 ± 0.86
STATE 3	40.03 ± 1.70	47.64 ± 1.44 *	53.78 ± 2.36 **, ***
ADP/O	2.53 ± 0.03	2.54 ± 0.02	2.54 ± 0.02
PR	101.2 ± 5.0	120.8 ± 2.7 *	136.5 ± 6.2 **, ***

Results shown are means ± SEM with n values in parentheses
RC, respiratory control ratio ; STATE 3, state 3 respiration rate (nat/mg protein/min) ; PR, phosphprylation rate (n mol/mg protein/min)
* $P < 0.05$; ** $P < 0.001$, as compared to control group
*** $P < 0.05$, as compared to TPN-G group

group. The PR was enhanced in both the TPN groups, and was more markedly in the TPN-V group than TPN-G group.

These results indicate that mitochondrial phosphorylative activity was enhanced significantly in the TPN groups, and that the enhancement was caused because of the marked decrease of EC in the TPN-V group.

Discussion

With the development of total parenteral nutrition (TPN), the number of malnutrished patients receiving TPN has increased. On the other hand, many authors have recentry reported the occurrence of complications associated with TPN[25,26,29], the most frequent of which are cholestatic jaundice and catheter sepsis. With the improvement of TPN solutions and infusion methods, these complications have decreased. But, despite many studies, the etiology of cholestatic jaundice or liver dysfunction in relation to TPN remains unclear.

We report that the liver mitochondrial phosphorylative activity, energy metabolism and keton body ratio (KBR) are altered under various forms of metabolic overload[32,35]. For example, hemorrhagic shock induces the decrease of liver mitochondrial phosphorylative activity[36], where as the endotoxin shock decreases energy charge level, but enhances mitochondrial phosphorylative rate[9].

In this study, we evaluated the effect of TPN on liver mitochodrial function in rats. EC was deteriorated in the groups infused with TPN solution, because the metabolic overload due to TPN leads to an increase in energy consumption. That is to say, because of glycogenesis due to the overload of glucose caused by TPN, energy was consumed, leading to a decrease in EC. Since intragastric hyperalimentation is more physiological, the deterioration of EC in the intragastric TPN group was less than that in the intravenous TPN group. Nordstrand et al studied the effect of TPN via portal vein and found that the side effects of transcaval TPN were less when the nutritional substrates were infused via the portal vein. Our findings also show that when the TPN solution was infused via the intragastric route (indirectly via the portal vein), there was less impairment of liver function with TPN. In contrast, the PR in the TPN groups was enhanced to compensate for the loss of EC.

In this experiment, after receiving TPN for 13 days, liver dysfunction such as jaundice and cholestasis was not perceived. But in the TPN group, despite evidence that liver function tests were normal, enhanced mitichondrial phosphorylative activity was observed during the early stage of TPN.

Finally, when the metabolic overload with TPN is prolonged the liver mitochondrial function is seriously impaired. Following that, liver dysfunction, such as are cholestatic jaundice and elevation of serum transaminase, may be induced. Long term TPN may thus induce liver failure.

To demonstrate this hypothesis, further evaluation of liver mitochondrial function with long term TPN is needed, including an evaluation of the differences of TPN administration route on liver mitochondrial function, i.e., via systemic vein or the portal vein.

In this study, we don't investigate the effect of liver mitochondrial function with a variety of compositions on the TPN solutions. So we consider that there is not such an effect but, it can not be concluded that differencies of the TPN solution do not infuluence the liver mitochondrial function at all. Making clear this question, similarly further studies will be necessary.

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和文抄録

成熟ラットにおけるTPNの肝ミトコンドリア機能に対する影響

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TPNの肝機能への影響を調べるために，肝 Energy charge，肝ミトコンドリア酸化的リン酸化能及び血清トランスアミラーゼ等の変化を検討した．

実験は240～250 gのSDラットを用い，右頸静脈から高カロリー輸液を投与した群（以下 TPN-V 群，n＝6），同様の高カロリー輸液を胃瘻から投与した群（以下 TPN-G 群，n＝5)及び上記2群と同カロリーの飼料を投与した群（以下コントロール群，n＝6)の3群に分け比較検討した．各群とも13日間 240 Kcal/kg/日のカロリーを投与した．しかし，最初2日間は 80 Kcal/kg/日（一日目），160 Kcal/kg/日（2日目)と漸増させていき，3日目以降に Full dose とした．実験開始14日目に屠殺し肝を摘出，肝ミトコンドリアを分離し肝 Energy charge，肝ミトコンドリア機能を測定した．結果は肝ミトコンドリア酸化的リン酸化能がコントロール群では 101.2±5.0 nmol/mg protein/min，TPN-G 群では 120.8±2.7 nmol/mg protein/min，そして TPN-V 群では 136.5±6.2 nmol/mg protein/min，であった．一方肝 Energy charge は各々0.906±0.006，0.889±0.008 そして 0.831±0.010 であった．これらの結果より TPN のカロリーの負荷が肝ミトコンドリア機能を元進させるが，肝 Energy charge を低下させることが分かった．